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Combined Effect of Low-Frequency Laser and Polyphenol Oxidase on Adhesive Activity of Escherichia coli Strains Isolated from Patients with Urinary Diseases.

Kolganova TV, Dzharadat T, Ermolaev AV, Osipova IG, Vasil'eva EA, Dalin MV, Doyle RJ.

Russian University of Peoples' Friendship, Moscow; Department of Microbiology and Immunology, Medical Center, University of Louisville, Kentucky, USA; L. A. Tarasevich State Institute of Standardization and Control of Biomedical Preparations, Moscow.

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The effects of polyphenol oxidase and low-frequency laser irradiation on adhesion of pathogenic Escherichia coli to human erythrocytes and buccal epithelial cells were studied. The maximum decrease in adhesive activity of these strains was observed after complex exposure to laser and enzyme.

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Effects of asparaginase and polyphenol oxidase on adhesive characteristics of microorganisms.

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We studied the effects of polyphenol oxidase and asparaginase on microorganism adhesion to buccal epithelial cells. These enzymes reduced adhesion of pathogenic microorganisms (uropathogenic and Escherichia coli, Salmonella enteritidis, Entamoeba spp., Influenza virus, Candida albicans, Streptococcus spp.) and had virtually no effect on adhesive characteristics of probiotic variants of Escherichia coli and Lactobacillus fermentum.

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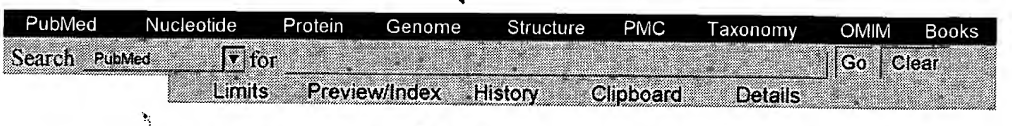

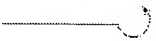

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1: Antimicrob Agents Chemother. 2000 Sep;44(9):2578-80. Related Articles, Links

Inhibitory effects of plant polyphenoloxidase on colonization factors of Streptococcus sobrinus 6715.

Cowan MM, Horst EA, Luengpailin S, Doyle RJ.

Department of Microbiology, Miami University, Oxford, Ohio 45056, USA. cowanmm@muohio.edu

Exogenously added polyphenoloxidase (EC 1.14.18.1), an enzyme which oxidizes tyrosine residues and is commonly found in many dietary components, abolished the aggregation of Streptococcus sobrinus 6715 by high-molecular-weight dextran. The enzyme decreased glucan-binding lectin and/or glucosyltransferase I activities.

PMID: 10952624 [PubMed - indexed for MEDLINE]

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1: Crit Rev Food Sci Nutr. 1981;15(1):49-127. Related Articles, Links

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Polyphenol oxidase and peroxidase in fruits and vegetables.

Vamos-Vigyazo L.

Polyphenol oxidases and peroxidases are among the most studied enzymes in fruits and vegetables. Owing to the deleterious effects of discoloration and off-flavor formation induced by their actions, these enzymes have not ceased to be a matter of concern to food technologists, while their versatility as catalyst and their diversity as protein present a challenge to the biochemist. This article gives an account on the present state of knowledge in this field. The occurrence of polyphenol oxidases and peroxidases in food and food raw materials, and their role and importance in food processing are briefly outlined. Results of biochemical research including catalytic properties, substrate specificity, susceptibility towards pH and temperature, action of inhibitors, isolation, purification, and characteristics of the enzymes are given, with special emphasis on recent achievements based on high resolution separation and isoenzyme techniques. Finally, the behavior of polyphenol oxidase and peroxidase in selected major groups of fruits and vegetables is discussed. Some contradictions found in the literature are pointed out and some questions that have not been given the necessary attention by researchers so far are mentioned.

Publication Types:
- Review

PMID: 6794984 [PubMed - indexed for MEDLINE]

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Partial purification and some properties of polyphenol oxidase extracted from litchi fruit pericarp

Jiang, Y-M., Zauberman, G., Fuchs, Y. (1997)
Postharvest Biology and Technology 10:221-228

Litchi (*Litchi chinensis* Sonn.) fruit peel polyphenol oxidase (PPO) was partially purified 21 fold by ammonium sulfate fractionation and gel filtration. Pyrogallol, catechol and 4- methylcatechol were good substrates for the enzyme; with no activity observed with chlorogenic acid, p-cresol, resorcinol, or tyrosine. The optimal pH for PPO activity was 7.0 with 4-methylcatechol, with the enzyme being most stable at pH 7.4. The enzyme was relatively temperature stable with maximum activity at 70°C and requiring a little less than 10 min at 90°C for 50% loss of activity. The Km and Vmax for the enzyme, with 4-methylcatechol, were 10 mM and $1.47x10^4$ units/min per mg protein respectively. The enzyme was not activated by SDS. Reduced glutathione, L-cystein, tropolone, thiourea, $FeSO_4$, and $SnCl_2$ markedly inhibited PPO activity, whereas $MnSO_4$ and $CaCl_2$ enhanced PPO activity. Data obtained in this study might help to better understand and control commercially, litchi peel browning.

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ACCESSION NO: 9156840
PROJ NO: NYC-149374 AGENCY: CRGO NY.C
PROJ TYPE: CRGO
START: 01 SEP 91 TERM: 31 AUG 95 FY: 1995 GRANT YR: 1993

INVESTIGATOR: Tingey, W. M.; Tingey, W. M.

PERFORMING INSTITUTION:
PLANT BREEDING
CORNELL UNIVERSITY
ITHACA, NEW YORK 14853

FUNCTION AND EXPRESSION OF POLYPHENOL OXIDASE

CONTRACT/GRANT/AGREEMENT NO: 91-37301-6571
GRANT YEAR: 1993

OBJECTIVES: PROJ. #9101665. Polyphenol oxidases (PPO) are copper metalloproteins which catalyze the oxidation of phenols to quinones at the expense of O(2). The PPO-generated quinones are highly reactive, electrophilic molecules which covalently modify and crosslink a variety of cellular constituents including nucleophiles of proteins such as sulfhydryl, amine, amide, indole and imidazole substitutents. A 59 kD PPOI in glandular trichomes on the leaf surface of wild tomato and potato species appears to be responsible for polymerization of glandular trichome exudate which results in insect entrapment. This PPO is the dominant protein and oxidative enzyme of these organs (ca. 4-80% of total trichome protein).

APPROACH: This proposal seeks to address the expression of 59 kD PPO by identifying cis-regulatory sequences controlling the high-level, epidermis and glandular trichom-specific expression of 59 kD PPO. The DNA encoding the tomato 59 kD trichome polyphenol oxidase gene will be isolated. A series of gene constructs utilizing pBI121 and 5' noncoding regions of the trichome PPO gene will be made to place Beta-glucuronidase under the control of sequences specifying the high-level, epidermal expressions of this gene. Identification of regulatory sequences for the 59 kD PPO may make possible the utilization of glandular trichomes for delivery of bioactive natural products to the leaf surface of crop plants.

PROGRESS: 9501 TO 9512
Polyphenol oxidases convert phenolic substrates to ortho-quinones whose secondary reactions are responsible for much of the oxidative browning evident in fruits and vegetables. However, an in planta function for PPOs has not been determined despite their wide distribution. Roles in plant defense, phenylpropanoid biosynthesis, plastidic oxygen regulation and electron transport have been hypothesized. To gain insight into PPO function(s), we introduced a chimeric antisense PPO cDNA into tomato. Oxidation of caffeic acid, the dominant o-diphenol of tomato foliage, was decreased 39-fold by antisense expression of PPO in transgenic tomato. Immuno-reactive PPO and PPO-specific mRNA and immunoreactive PPO were notdetectable in the transgenic plants. Growth and development were phenotypically normal under greenhouse conditions. However, epidermal leucoplasts displayed an abnormal morphology in antisense plants. PPO-containing protein bodies conspicuous in nontransformed plants were absent or reduced in antisense PPO plants and plastids did not label with antibodies directed

against PPO. However, insect herbivore performance is significantly improved on transgenic tomatoes possessing antisense PPO constructs, indicating that PPO may play a role in plant defense against herbivores.

PUBLICATIONS: 9501 TO 9512

Thipyapong, P., Hunt, M.D., and J.C. Steffens (1995). Systemic wound induction of polyphenol oxidase in potato. Phytochemistry 40:673-676. Hunt, M.D. (1995). Cloning, expression, and functional analysis of polyphenoloxidases. PhD Thesis, Cornell University, 213 pp.

SUBFILE: CRIS

Asparaginase

Scott Hamilton

Function of the Enzyme

The general functon of the enzyme ***Aspariginase*** is to catalyse the reaction of

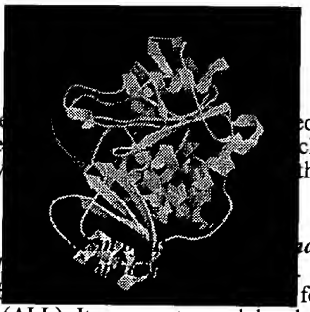
$$\text{L-asparigine} + H_2O \xleftarrow{\text{aspariginase}} \text{L- aspartate} + NH_3$$

The EC number of this particular enzyme is **3.5.1.1** Aspariginase **(1)**

Protein Characteristics

The enzyme belongs to the protein family: nitrilase, it has a molecular weight of 38,895 Daltons. The amino acid sequence is **tggt**. **(2)**

Protein Structure



(3)

Current Research

The asparaginase[illegible]d by the computer generated images. It is classified as an alpha-be[illegible]classified as a three-layer alpha-beta-alpha sandwich. The topology[illegible]he homologous superfamily is classified as a hydrolase. **(3)(4)(5)**

Insight into the [illegible]duced depletion of antithrombin III in treatment of childhood

Asparaginase is [illegible]for the treatment of childhood acute lymphoblastic leukemia (ALL). It appears to work by cleaving asparagine to give aspartic acid and ammonia and thus depleting free asparagine in the blood. Evidence suggests, however, that it can cause a predisposition to thromboembolic problems by significantly decreasing serpin and antithrombin III plasma levels. Human plasma and whole blood showed no loss of antithrombin III activity when treated with theraputic levels of asparaginase. **(6)**

The L-Asparagine operon of Rhizobium etli contains a gene encoding an atypical asparaginase.

Sequence analysis for the L-asparagine operon of Rhizobium etli was cloned and sequenced. The analysis demonstrated four adjacent open reading frames(ansR, ansP, ansA, and ansB) which coded for a transcriptional repressor, a permease, a thermolabile asparaginase, and an aspartase, respectively. The product of the R. etli ansA gene showed a different sequence than previously reported microbial asparaginases, which suggests that it is an atypical asparaginase that may have come form another source other than bacteria or yeast asparaginases. **(7)**

may have some form another source other than bacteria or yeast asparaginases. (7)

Pegylated asparaginase (Oncaspar) in children with ALL.

Children who would have normally have been treated with native E. coli. asparaginase in induction therapy were switched according to protocol were switched or started on pegylated asparaginase and monitored closely because of observed adverse side-affects of the native asparaginase. Toxicity was low in comparison with the native enzymes, and no allergic reactions were seen. **(8)**

References (obtained Feb. 2001)

1) http://www.expasy.ch.cgi-bin/niceprot.pl?P46011. NiceProt View of SWISS-PROT: P46011
2) http://srs.ebi.ac.uk/srsbin/cgi-bin/wgetz?-e+[BRENDA-ECNumber:'3551']
3) Protein Data Bank, http://www.rcsb.org/pdb/index.html, Structure Explorer http://www.rcsb.org/pdb/cgi/explore.cgi?job=graphics&pdbId=1AGX&page=&pid=123889832
4) CATH (Classification, Architecture, Topology, and Homologous Superfamily of Proteins), http://www.biochem.ucl.ac.uk/bsm/cath_new index.html, http://www.biochem.ucl.ac.uk/bsm/cath_new/class3/40/50/40/index.html
5) http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=Text&DB=PubMed
6) Insight into the mechanism of...http://www.ncbi.nlm.nih.gov/PubMed/. Authors: Bushman, JE, Palmieri, D, Whinna, HC, Church, FC. , Department of Chemistry, University of North Carolina at Chapel Hill, USA. Information obtained February 2001.
7) The L-asparagine operon.... http://www.ncbi.nlm.nih.gov/PubMed/ Authors: Ortuno, OL, Duran-Vargas, S, Department of Biotechnology, University of Mexico, Mexico. Information obtained February 2001
8) Pegylated asparaginase(Oncaspor) in ... Journal of Haematol, Aug. 2000, pg 379-84. http://www.ncbi.nlm.nih.gov/PubMed/ , Authors: Mueller HJ, et al. , University of Munster, Germany. Information obtained Feb. 2001

Polyphenol Oxidase

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Catalog Index

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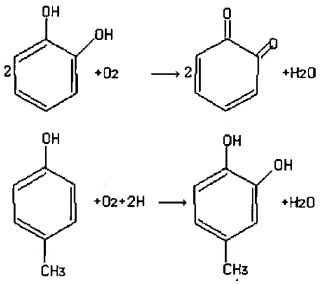
I.U.B.: 1.14.18.1

Tyrosinase
monophenol, dihydroxyphenylalanine: O_2 oxidoreductase

Polyphenol oxidase (tyrosinase) (TY) is a bifunctional, copper-containing oxidase having both catecholase and cresolase activity (Malmström and Rydén 1968):



Jolley *et al.* (1974) refer to it as an oxygen and 4 electron-transferring phenol oxidase. It is responsible for browning reactions throughout the phylogenetic scale.

Although a tyrosinase from *Neurospora crassa* has been purified (Fling *et al.* 1963), most work has been done with the mushroom enzyme, even though yields and consistency are poor; its multiplicity was shown by Smith and Krueger (1962). Bouchilloux *et al.* (1963) obtained four enzymes. See review by Nelson and Mason (1970).

Characteristics of Polyphenol Oxidase from Mushroom:

Molecular weight: 128,000 (Duckworth and Coleman 1970).

Composition: The enzyme is a tetramer containing four gram atoms of copper per molecule (Jolley *et al.* 1974), and two binding sites for aromatic compounds including phenolic substrates. There is also a distinctly different binding site for oxygen, the copper site (Duckworth and Coleman 1970). The copper is probably in the cuprous state; inactivation of the enzyme is associated with increase in Cu^{2+}. (Kertész *et al.* 1972). The amino acid composition has been determined. Extensive structural studies have been reported by Jolley *et al.* (1969); and Duckworth and Coleman (1970). See also Jolley *et al.* (1972, 1973, and 1974).

Optimum pH: 6.0-7.0.

Extinction coefficent: $E^{1\%}_{280}$ = 24.9 (immediately after purification) (Duckworth and Coleman 1970).

Inhibitors: Compounds that complex with copper. The enzyme is also inhibited competitively by benzoic acid with respect to catechol and by cyanide with respect to oxygen (Duckworth and Coleman 1970).

Activity: Polyphenol oxidase is an oxygen transferring enzyme. Besides using O_2 to catalyze the dehydrogenation of catechols to orthoquinones and the orthohydroxylation of phenols to catechols, a peroxidase activity has been reported on by Strothkamp and Mason (1974). Kinetic studies have been reported by Kertész *et al.* (1971). See also the review by Malmström and Rydén.

Specificity: A large number of parasubstituted catechols areoxidized (Duckworth and Coleman 1970).

Stability: The lyophilized preparation is stable for 6-12 months when stored at 5°C.

Assay

Method: Polyphenol oxidase oxidizes tyrosine to dihydroxyphenylalanine which in turn is oxidized to o-quinone. The latter is accompanied by an increase in absorbance at 280 nm. The rate of increase is proportional to enzyme concentration and linear during a period of 5-10 minutes after an initial lag. One unit causes a change in absorbance at 280 nm of 0.001 per minute at 25°C, pH 6.5 under the specified conditions.

Reagents

- 0.5 M Potassium phosphate buffer, pH 6.5
- 0.001 M L-Tyrosine

Enzyme

Dissolve at a concentration of 1 mg/ml in reagent grade water. Dilute further in reagent grade water to a concentration of 200-400 units/ml.

Procedure

Adjust the spectrophotometer to 280 nm and 25°C. Pipette into each cuvette as follows:

0.5 M Phosphate buffer, pH 6.5	1.0 ml
0.001 M L-Tyrosine	1.0 ml
Reagent grade water	0.9 ml

Oxygenate this reaction mixture by bubbling oxygen into cuvettes through a capillary tube for 4-5 minutes. Transfer cuvettes to the spectrophotometer and record A_{280} for 4-5 minutes to achieve temperature equilibration and to establish blank rate, if any. Add 0.1 ml of appropriately diluted enzyme and record A_{280} for 10-12 minutes. Determine $?A_{280}$ from the linear portion of the curve. A non-linear "lag" of 2-3 minutes can be expected.

Calculation

$$\text{Units/mg} = \frac{\Delta A_{280}/\text{min} \times 1000}{\text{mg enzyme in reaction}}$$

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Effects of asparaginase and polyphenol oxidase on adhesive characteristics of microorganisms.

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We studied the effects of polyphenol oxidase and asparaginase on microorganism adhesion to buccal epithelial cells. These enzymes reduced adhesion of pathogenic microorganisms (uropathogenic and Escherichia coli, Salmonella enteritidis, Entamoeba spp., Influenza virus, Candida albicans, Streptococcus spp.) and had virtually no effect on adhesive characteristics of probiotic variants of Escherichia coli and Lactobacillus fermentum.

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Plasma/intestinal concentration patterns suggestive of entero-portal recirculation of amino acids: effects of oral administration of asparaginase, glutaminase and tyrosinase immobilized by microencapsulation in artificial cells.

Chang TM, Lister C.

Artificial Cells and Organs Research Centre, Faculty of Medicine, McGill University, Montreal, P.Q., Canada.

This study suggests the presence of an entero-portal recirculation of amino acids. Endogenous sources of amino acids are secreted at high concentration into the small intestine. Most of the amino acids are absorbed as the content passes down the small intestine. Plasma amino acid concentrations are on the average only 1-5% of the concentrations in the duodunum. This is true even in rats on 24 hours of water and sugar with no exogenous sources of amino acids. For example, the PLASMA:DUODENUM concentrations (mumole/litre) are: Asparagine 37:7164, Tyrosine 94:9579, and glutamine/histidine 409:9708. This entero-portal recirculation of amino acids means the potential of a method for specific depletion of body amino acids by oral ingestion of bioreactants like immobilized enzymes. Preliminary studies used artificial cells to immobilize asparaginase,glutaminase and tyrosinase by microencapsulation. Six hours after 1 oral administration, asparagine, glutamine and tyrosine in the ileum were lowered to 10% of the level of the control. Artificial cells containing no enzymes were used as the control.

PMID: 3150943 [PubMed - indexed for MEDLINE]

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Modulation of lymphocyte proliferation by enzymes that degrade amino acids.

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In a previous study we demonstrated thirteen amino acids to be essential and two to be partially essential for lymphocyte proliferation. Arginine is one of the essential amino acids, and the highly purified arginase strongly inhibited lymphocyte proliferation. The modulation of lymphocyte growth by various amino acid-degrading enzymes was studied. Peripheral lymphocytes were cultured in RPMI 1640 with or without amino acid-degrading enzyme for 72 h. A total of 17 commercial L-amino acid-degrading enzymes were studied. At 10 micrograms/ml, both lysine decarboxylase and asparaginase completely inhibited lymphocyte proliferation, arginase resulted in 78% inhibition and tyrosinase 57% inhibition. Other enzymes inhibited less than 20% lymphocyte proliferation; they included alanine dehydrogenase, arginine decarboxylase, aspartase, glutamic decarboxylase, glutamic dehydrogenase, glutaminase, histidase, histidine decarboxylase, leucine dehydrogenase, phenylalanine decarboxylase, phenylalanine hydroxylase, tryptophanase, and tyrosine decarboxylase. All four enzymes that strongly inhibited lymphocyte proliferation degraded amino acids that are essential for lymphocyte growth.

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Kinetic analysis of microbial adhesion.

Cowan MM.

Department of Biochemistry, Microbiology, and Molecular Genetics, University of Rhode Island, Kingston 02881, USA.

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Response of microbial adhesives and biofilm matrix polymers to chemical treatments as determined by interference reflection microscopy and light section microscopy.

Marshall PA, Loeb GI, Cowan MM, Fletcher M.

Center of Marine Biotechnology, University of Maryland, Baltimore 21202.

PubMed Services

The polymers involved in the adhesion of Pseudomonas fluorescens H2S to solid surfaces were investigated to determine whether differences between cell surface adhesives and biofilm matrix polymers could be detected. Two optical techniques, i.e., interference reflection microscopy (IRM) and light section microscopy (LSM), were used to compare the responses of the two types of polymer to treatment with electrolytes, dimethyl sulfoxide (DMSO), and Tween 20. To evaluate initial adhesive polymers, P. fluorescens H2S cells were allowed to attach to glass cover slip surfaces and were immediately examined with IRM, and their response to chemical solutions was tested. With IRM, changes in cell-substratum separation distance between 0 and ca. 100 nm are detectable as changes in relative light intensity of the image; a contraction of the polymer would be detected as a darkening of the image, whereas expansion would appear as image brightening. To evaluate the intercellular polymer matrix in biofilms, 3-day-old biofilms were exposed to similar solutions, and the resultant change in biofilm thickness was measured with LSM, which measures film thicknesses between 10 and 1,000 microns. The initial adhesive and biofilm polymers were similar in that both appeared to contract when treated with electrolytes and to expand when treated with Tween 20. However, with DMSO treatment, the initial adhesive polymer appeared to contract, whereas there was no change in thickness of the biofilm polymer. These results indicate that both polymers bear acidic groups and thus act electrostatically with cations and are able to enter into hydrophobic interactions.(ABSTRACT TRUNCATED AT 250 WORDS)

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Role of sialic acid in the kinetics of Streptococcus sanguis adhesion to artificial pellicle.

Cowan MM, Taylor KG, Doyle RJ.

Evaluation of the kinetics of adhesion of Streptococcus sanguis 10556 to saliva-coated hydroxylapatite revealed that sialic acid played a role in the formation of a stable cell-substratum complex. In a previous paper (M. M. Cowan, K. G. Taylor, and R. J. Doyle, J. Dent. Res. 65:1278-1283, 1986) the adhesion was found to take place in two distinct stages: a reversible equilibrium, probably governed by long-range forces, followed by a transition to higher-affinity binding. In the present study, artificial pellicle was treated with neuraminidase, and kinetic adsorption and desorption experiments with S. sanguis were conducted. The depletion of sialic acid from pellicle decreased the initial adsorption rate constant only slightly. The rate constant describing the initial desorption was unaffected. However, no transition to the second (high-affinity) association occurred. While S. sanguis desorption from control pellicles exhibited two sequential rates, with the second rate being approximately 10 times slower than the first, all desorption from sialo-deficient pellicles occurred at one rate that was equivalent to the initial rate constant for control desorption. The cells did not reach an equilibrium with the sialo-deficient pellicle, even after 6 h. Competing sialic acid did not decrease the rate or extent of adsorption, but desorption occurred to a greater extent when cells had adsorbed in the presence of sialic acid. These data suggest that sialic acid plays little role in the initial association of cell and pellicle but that it is necessary for the transition to high-affinity binding and the concomitant decreased propensity to desorb.

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Kinetic analysis of Streptococcus sanguis adhesion to artificial pellicle.

Cowan MM, Taylor KG, Doyle RJ.

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Studies of equilibria between Streptococcus sanguis and artificial pellicle have suggested that there are multiple binding sites for the organism. In the present study, adhesion of S. sanguis to saliva-coated hydroxylapatite was examined by means of kinetic methods. Cell-pellicle complex formation was measured from initiation of binding to equilibrium: Rate constants were calculated for forward reactions (adsorption) and reverse reactions (desorption). Initial binding obeyed reversible, first-order kinetics, whereas desorption of bound cells followed biphasic kinetics. Initial desorption proceeded approximately ten times faster than the slower second rate. The results are consistent with the mechanism C + P reversible CP* in equilibrium with CP in which CP* represents the reversible equilibrium that shifts at a discrete rate to the high-affinity CP state. Thus, the biphasic binding behavior that has been previously deduced from equilibrium studies may be attributed to a time-dependent shift from close apposition to pellicle, stabilized by low-specificity forces, to a higher-affinity binding.

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Studies of enzyme-catalyzed modification of proteins. I. Tyrosinase-catalyzed modification of asparaginase.

Tokushige M, Moriya F.

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Asparaginase [EC 3.5.1.1.] of Escherichia coli, an anti-tumor enzyme, was inactivated in a time-dependent fashion by mushroom tyrosinase [EC1.14.18.1.]. The inactivation did not proceed, however, when heat-inactivated tyrosinase was used. Exculusion of the atmospheric oxygen or addition of diethyldithiocarbamate, a copper selective chelating agent, prevented the inactivation. The difference absorption spectrum of tyrosinase-inactivated asparaginase versus intact asparaginase exhibited the appearance of marked absorption peaks at 300 and 350 nm. These results indicate that the tyrosyl residue(s) of asparaginase, which is essential for the activity is enzymatically modified by tyrosianes.

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